

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11544 Capn13 EXDEL

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (μL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20μM) comF	0.5
Primer 2. (stock concentration is 20μM) wtR	0.5
Primer 3. (stock concentration is 20μM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	15.00 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90
1. CR_Capn13_comF	GCCCCCTAATACCTACACCACC	Estimated Running Time: 90 min.
2. CR_Capn13_wtR*	GTGACTTCCCATGTGAGATCAGG	Primer Combination Band (bp) Genotype
3. CR_Capn13_mutR	CATGCTGTTCTATTCTACATCACAGTG	1 & 2, 1 & 3 225, 1195 wildtype
		1 & 3 415 mutant

Electrophoresis Protocol:

Allele Description: Exon 4 ENSMUSE00000343189 and flanking splicing regions were constitutively deleted from the Capn13 Gene ENSMUSG00000043705 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 780bp deletion from Chr 17: 73672620 - 73673399 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)

